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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
WALICKA, MALGORZATA A	
ART UNIT	PAPER NUMBER
1652	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/808,717	SAN ET AL.	
	Examiner	Art Unit	
	Malgorzata A. Walicka	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 June 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *27-33* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *27-33* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application June 29, 2007, after advisory action of May 3, 2007. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office Action has been withdrawn pursuant to 37 CFR 1.114.

2. Declaration of Dr. G. N. Bennet under 37 CFR §1.132 filed June 29, 2007 is acknowledged.

3. Applicants also filed an amendment to Table 2 of the specification. The current response does not comprise any amendment to the claims. Claims 27-33 are pending and under examination.

Detailed Action

Objections

The amended claims contain changes in spelling which are not properly marked.

Please correct spelling of the names of genes throughout the claims. The enzyme pantothenate kinase is abbreviated PANK and its gene is abbreviated *panK;* see, for example *pta, atf.*

In addition, the amendment to Table 2 is not proper, because it does not visualize additions and deletions made.

Please note that claim 31 does relates to utility of the increase of the CoA flux, because that increase is used for production of ester, in the instant case acetate ester.

Rejections

35 USC section 112, second paragraph

Claim 27 is rejected as indefinite as to what is the purpose of the increase of the CoA flux by transforming a cell with genes (i), (ii), and (iii). Claim 32 tries to state the purpose of increasing the flux of CoA by adding "thereby increasing production of isoamyl acetate". The latter phrase, however, lacks the antecedent in the claim. The examiner suggests using the closed language and limiting the increase in CoA to the purpose of producing isoamyl acetate.

35 USC section 112, first paragraph

Written description

Claims 27-33 have been rejected in the Office Action of 01/29/07 (previous action).

Rejection of claim 31 for new matter is withdrawn, because applicants' arguments are found persuasive.

The declaration of Dr. George N. Bennet under 37 CFR §*1.132 filed on June 29, 2007 is sufficient to overcome the rejection of claims 27-33 based upon insufficient description of a bacterial cell and pantothenate kinase gene, pyruvate dehydrogenase gene, and alcohol acetyl transferase gene used for its transformation.

Claim 31 is rejected because certainly not any bacterium may be successfully transformed to attain the goal of an increase in conversion of an alcohol to an ester) as claimed in claim 31, simply because bacteria are very versatile in their metabolism. **Any bacteria does not produces any alcohol, the conversion of which into ester may** be increased by an increase in CoA flux as recited by claim 27. Please note that claim 27 limits esters to acetate esters, in view of the fact that it recites using alcohol **acetyl** transferase gene for transformation. Acetate esters are not recited by claim 31. Addition of an alcohol as a substrate for production of the ester in the bacterium non-producing said alcohol is necessary, but may be toxic for the transformed host. In result of this toxicity production of the ester may be not possible. Thus, the alcohol recited in claim 31 may not be any alcohol but should be identified. In the case at hand Applicants have not described any bacterium producing any alcohol that after transformation with genes i), ii) and iii) of claim 27, i.e., after increasing CoA flux, increased conversion of the alcohol into ester. E. coli, the bacterium used by applicants, does not produce any alcohol (i.e., all alcohols) and their esters itself. In the instant application, to make E. coli a producent of isoamyl acetate applicants had to transform it with alcohol acetyl transferase gene and cultivate it in the presence of isoamyl alcohol. Thus, the definite alcohol was provided in the growth medium, and acetate ester was produced because E.coli was transformed with acetyl transferase. However, teaching conversion of isoamyl alcohol by acetyl transferase into isoamyl acetate does not teach conversion of any alcohol into its acetate ester. All together, applicants have failed to sufficiently describe the claimed invention in such full, clear,

concise and exact terms that a skilled artisan would recognize applicants were in possession of the claimed invention at the time the application was filed.

Rejection for new matter

Claims 28, 32 and 33 are rejected for lack of written description of a bacterium cell having reduced activity of *ackA* or *pta*. Applicants teach they used E. coli YBS121 double mutant *ackA-pta* that has both genes inactivated. Neither the specification nor the claims as originally filed teach a bacterium cell having reduced activity of *ackA* gene or *pta* gene separately. This rejection is for new matter.

Response to applicant's traverse

In the recent REMARKS, page 5/10, Applicants argue, "The YBS 121 (Δ*ackA* - Δ*pta*) is not a 'mutant' strain rather it is a strain generated by chromosomal integration that has 'mutant copies acetate kinase (*ackA*) and phosphoacetyltransferase'(*pta*) p.15 ¶ [57]. Thus each gene *ackA* and *pta* was intentionally mutated to generate the double-mutant YBS 121. Therefore, Applicants describe Δ*ackA* and Δ*pta*. Either Δ*ackA* or Δ*pta* could be intentionally mutated, or as one example, both could be intentionally mutated."

This argument of applicants is not found persuasive for the following reasons.

1. When a strain is generated by chromosomal integration that has "mutant copies acetate kinase (*ackA*) and phosphoacetyltransferase(*pta*)" is still a mutant strain. In the next sentence applicants use the term "double mutant". A double mutant is still a

mutant. However, a double mutant is not a single mutant. Biologically, a strain mutated in *ackA* is not a strain mutated in *pta,* and a (*ΔackA* - *Δpta)* strain is not the same as *ΔackA* strain and is not the same as *Δpta* strain.

2. Indeed, one can intentionally mutate any gene, *ackA* or *pta*, or other gene or two or five of them. However, applicants received as a gift the strain that has mutated both genes *ackA* and *pta,* and this very mutant was used in the claimed method. Nowhere in the disclosure one can find "either ackA or pta can be mutated" or an equivalent language.

Scope of enablement

Claim 27-33 were rejected for reasons explained in the previous action. The declaration of Dr. George N. Bennet under 37 CFR §1.132 filed on June 29, 2007 is sufficient to overcome the rejection of claims 27-33 based upon scope of enablement made in the previous action; see the above withdrawal of rejection for lack of written description.

Claim 31 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the method of production, and increasing the production of isoamyl acetate in E. coli transformed with genes (i), (ii) and (iii), and growing the transformants in the medium supplemented with pantotenic acid, does not provide a reasonable enablement for production of acetate ester of any alcohol.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention

commensurate in scope with claim 31. The claim is broader than the enablement provided by the disclosure with regard to the extremely large and versatile genus of chemicals compounds, i.e., acetic acid esters, production of which is to be increased by transformation of any bacterial cells with genes (i), (ii) and (iii).

The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Otherwise, undue experimentation is necessary to make the claimed invention. Factors to be considered in determining whether undue experimentation is required, are summarized *In re* Wands [858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)]. The Wands factors are: (a) the nature of the invention, (b) the breadth of the claim, (c) the state of the prior art, (d) the relative skill of those in the art, (e) the predictability of the art, (f) the presence or absence of working example, (g) the amount of direction or guidance presented, (h) the quantity of experimentation necessary.

The nature and breath of the claimed invention encompasses in production of any acetic acid ester by any bacterium transformed with pantothenate kinase gene, pyruvate dehydrogenase gene, and alcohol acetyl transferase gene, wherein the transformed bacterium is cultivated in the presence of pantothenic acid.

While methods of producing esters by engineered bacteria are known in the art, and skills of the artisans well developed, no one is able to make any acetate ester without indicating what alcohol takes part in estrification. No bacteria produces any alcohol (i.e., all alcohols) as required by the claim. Thus, one having skills in the art who would like to make the claimed invention is forced to experimentation that is not

routine, and has a low probability of success as long as the applicants do not identify a suitable alcohol that is produced by the bacterium or which has to be supplemented to the culture medium. Providing for production of isoamyl acetate in E. coli grown in the presence of isoamyl alcohol does not provide an instruction or guidance how to make the claimed invention because the isoamyl alcohol does not identify any alcohol, and the fact that certain concentration of isoamyl alcohol in the culture medium are not toxic to E. coli does not mean that any alcohol is not toxic to any bacterium. The examiner concludes that without a further guidance on the part of applicants in regarding the alcohol that is to be converted into ester, experimentation left to those in the art is improperly extensive and undue.

3.4. 35 USC 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 27-33 are rejected as being unpatentable over of San et al. (Metabolic Engineering through Cofactor Manipulation and Its Effects on Metabolic Flux Redistribution in *Escherichia coli*, Metabolic Engineering, February 27, 2002, 4, 182-

192, included in the Information Disclosure Statement), Song and Jackowski (Cloning, Sequencing and Expression of the Panthothenate Kinase (*coaA*) Gene of E. coli, J. Bacteriol, Oct. 1992, 174, 6411-6417; copy enclosed), Voet et al. (Biochemistry, second Edition, 1995, John Wiley & Sons, Inc, pp. 543-548, mailed to applicants with the previous action), and Yang et al. (Effect of Inactivation of *nuo* and *ackA-pta* on Redistribution of Metabolic Fluxes in *Escherichia coli*, Biotech. Bioeng. 1999, 65, 291-297, mailed to applicants with the previous action), and routine practice in the field of metabolism engineering.

Claim 27-30 are directed to a method that increasing coA flux in a bacterial cell comprising transforming the cell with pantothenate kinase gene, pyruvate dehydrogenase gene, and alcohol acetyl transferase gene, and in case of claim 29 in addition by reducing activity of ackA or pta or ackA-pta genes. The claims are directed to a method of manipulating the metabolism of the bacterial by an increase in CoA flux through the acetyl-CoA node. Claim 31 is directed to a method of producing any acetate ester and claim 32-33 to production of isoamyl acetate.

San et al. 2002, in Fig. 6a teach that CoA is synthesized in a series of reaction which start with phosphorylation of pantotenic acid by pantotenate kinase (panK). San et teach in Fig. 6b that CoA and pyruvate are used for synthesis of acetyl-CoA by pyruvate formate lyase (under anaerobic conditions). Actually, under aerobic conditions, as taught by Biochemistry handbook on page 543 the synthesis of acetyl–CoA is performed by **pyruvate dehydrogenase, a multicomplex enzyme**, page 543, Fig. 19.6, and the text under the figure. Next, Fig. 6b of San et al. 2002, teaches that

alcohol acetyl transferase (AAT, in the instant application ATF) uses acetyl-CoA as a source of acetyl in synthesis of acetate ester from an alcohol. Thus, San et al, 2002, teach the flux of CoA from its synthesis starting with pantotenate kinase, through pyruvate dehydrogenase to acetyl- CoA and San et al 2002 teach further the transfer of acetyl group from acetyl-CoA to alcohol by alcohol acetyl transferase. Yang et al 1999 teach in Fig. 1 phosphoacetyltransferase (*pta*) and further acetate kinase (*ackA*) use acetyl-CoA for phosphorylation and production of acetate. Thus, these last enzymes compete in acetyl Co-A node with alcohol acetyl transferase for acetyl group of acetyl-CoA. Cutting out *pta –ackA* pathway would divert acetyl-CoA to its node. Yang et al used E. coli mutant having inactivated *pta –ackA* that produced acetyl–CoA but did not produced acetate; see Table IV, page 295.

Song and Jackowski 1992 teach that the regulation of pantothenate kinase step is the most important determinant of the CoA synthesis rate in E. coli (bacterial cell) and that panthotenate kinase is feedback inhibited by CoA; see introduction line 13. Biochemistry handbook teaches on page 547 that pyruvate dehydrogenase is inhibited by acetyl-CoA.

Song and Jackowski also teach transformation of E. coli (bacterial cell) with its panK gene using a multicopy plasmid, and cultivate the transformants in the medium containing an excess of 60μM extracellular pantothenate; see page 6415, left column under **Physiological consequences of pantothenate kinase overexpression and Fig. 7**.

San et al. 2002 teach transformation of E. coli with a plasmid encoding ATF2 and cultivating transfromants in a medium supplemented with 10 mM isoamyl alcohol. Because E. coli itself does not synthesize isoamyl alcohol, thus isoamyl acetate would be only formed when isoamyl alcohol is added externally; see the paragraph bridging page 190 and 191.

Taking into account the above described teachings one having skills in the art, who would like to increase CoA flux in a E. coli (bacterial cell) or efficiently produce isoamyl acetate in bacterial cell would transform it with a multicopy panK plasmid and grow it in the presence of extracellular pantothenate as Song and Jackowski 1994 did. Furthermore one having skills in the art would transform E. coli with, PDH gene to increase production of acetyl CoA, and finally one having skills in the art would transform bacterial cell with ATF and grow them in the presence of isoamyl alcohol as San et al. 2002 did. Transformation with panK would increase concentration of CoA in the cell in the first place. Transformation of the cell with PDH would increase the use of CoA for synthesis of acetyl-CoA. Thus, panK would not be inhibited by CoA, which would fuel further synthesis of CoA by panK. Use of acetyl-CoA by ATF would release feedback inhibition of PDH by Acetyl-CoA which would promote its further synthesis from pyruvate and CoA, and in this situation also further synthesis of CoA. Finaly, using cells that have inactivated *pta –ackA* would increase the amount of acetyl–Co avalilable for ATF in acetate ester synthesis.

Probability of success in transformation with the genes as very high as evidenced by Song and Jackowski for panK, and by San et al for ATF; as well as, because at the

time if invention transformation of bacterial cell with endogenous or exogenous genes was a routine practice. All claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed with no change in their respective functions, and the combination would yielded predictable results to one of ordinary skill in the art at the time of the invention. Thus, the claimed invention was within the ordinary skill in the art to make and use at the time it was made, and was as a whole *prima facie* obvious.

Applicants' attention is turned to the fact that the prima facie obviousness is supported by Dr. Bennet Declaration filed June 29, 2007 in which he writes, "Overexpression of pantothenate kinase will increase coenzyme A production in **all** characterized bacterial species" and further, "Thus, expression of pantothenate kinase, pyruvate dehydrogenase, and alcohol acetyl transferase described in claims 27-33 will increase CoA flux to secondary metabolites in all bacteria."

In conclusion, the state of art at the time of invention makes the invention obvious.

Response to Applicants arguments

Applicants traverse previous version of obviousness rejection providing Secondary Indications of Non-obviousness REMARKS of June 29, 2007, page 8/10:

> "As noted by Vadali, et al. (Metab. Eng. 6:113-9 (2004), at page 138)[1], 'It was found that the intracellular CoA/acetyl AcoA could be increased **only** with the simultaneous overexpression of pantotenate kinase **and supplementation**

of pantothenic acid. Since E. coli normally secretes out excess pantothenic acid, it might be logical to assume that the availability of precursor will not be rate limiting. On the contrary, the **supplementation of pantothenic acid is essentially and necessary** for CoA/acetyl-CoA manipulation[emphasis in Remarks].' Thus, at the time of filing, it was unexpected that bacteria would require supplementation with pantothenic acid, and, without supplementation increased CoA flux would not be achieved."

This argument, already raised after final rejection, was addressed by the examiner in her Advisory Action of May 3, 2007, and is repeated below with minimal modifications. In the above rejection for obviousness the examiner uses article by Song and Jackowski (1992) who teach transformation of E. coli (bacterial cell) with its panK gene using a multicopy plasmid. Song and Jackowski cultivate the transformants in the medium containing an excess of 60μM extracellular pantothenate; see page 6415, left column under **Physiological consequences of pantothenate kinase overexpression**. Thus, the art teaches that in case when many copies of panK are expressed in bacteria providing additional precursor in the medium is necessary for achieving higher synthesis of CoA. In general, one having skills in the art expects, when expressing many copies of a gene, that increasing synthesis of a product of the encoded enzyme requires an increase in precursor availability. This expectation was the rationale of experiments presented in Vadali, et al. (Metab. Eng. 6:113-9 (2004).

The Vadali article describes, among others, the data on E. coli transformed with panK gene on a multicopy plasmids; the same plasmids as used in the instant

application. The quoted passage of Vadali et al. originates from the **Conclusion** section of the article. The passage content is not in accord with the logic and results of experimentation described in the article in the section **Results and Discussion** *Characterization of DH10B (pUC19) and DHN(pRV380) in aerobic shake fasks*, page 135. In this section Vadali et al. present results for E. coli transformed with a plasmids comprising **high copy number of E. coli panK genes**. This overexpression, however, did not lead to an increase in the intracellular CoA/acetyl-CoA levels. Although the authors noted that not transformed E. coli contain excess amount of pantothenate acid and secrets it to the extracellular medium (right column of page 135, second line of the second paragraph), the authors also concluded (further in the same paragraph), as a skilled artisan would, "With the **overexpression of the panthothenate kinase, the availability of panthotenic acid might be rate limiting.** To test the requirement of concentration of pantothenic acid, a doseage study was performed... The results are shown in Fig.1." The results suggest that ~50 uM of pantothenic acid concentration in the cell culture medium is sufficient **to saturate the enzymatic activity of overexpressed pantothenate kinase** resulting in highest intracellular acetyl-CoA levels [emphasis added by the examiner]." **Please note that Song and Jackowski 1992 used in their studies of overexpression of panK in E. coli the concentration 60** uM pantothenic acid; see Fig. 7.

Applicants' attention is turned to the fact that if E. coli were transformed with one or two additional copies of panK, the supplementation would have been probably not necessary. In summary, at the time of filling, it was obvious that bacteria comprising

many copies of panK gene would require supplementation with pantothenic acid to increase the CoA flux through the acetyl CoA node. **Thus the supplementation has no feature of novelty.**

Conclusion

- All claims are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Malgorzata A. Walicka whose telephone number is (571) 272-0944. The examiner can normally be reached on Monday-Friday from 10:00 a.m. to 4:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can be reached on (571) 272-0928. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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